PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 October 2000 (13.10.00)	
International application No. PCT/US00/04326	**Applicant's or agent's file reference** L0461/7057WO
International filing date (day/month/year) 18 February 2000 (18.02.00)	**Priority date** (day/month/year) 22 February 1999 (22.02.99)
Applicant CHIARI, Rita et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 August 2000 (15.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
L0461/7057WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 04326	18/02/2000	22/02/1999
Applicant		
LUDWIG INSTITUTE FOR CANCER RESEARCH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 3

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]S 00/04326

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 G01N33/566 A61K38/17 C07K16/28
A61K39/395 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOYD A W ET AL: "ISOLATION AND CHARACTERIZATION OF A NOVEL RECEPTOR-TYPE PROTEIN TYROSINE KINASE (HEK) FROM A HUMAN PRE-B CELL LINE" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 267, no. 5, 15 February 1992 (1992-02-15), pages 3262-3267, XP000615518 ISSN: 0021-9258 the whole document --- -/--	1-64

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hix, R

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WICKS I P ET AL: "MOLECULAR CLONING OF HEK, THE GENE ENCODING A RECEPTOR TYROSINE KINASE EXPRESSED BY HUMAN LYMPHOID TUMOR CELL LINES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 89, 1 March 1992 (1992-03-01), pages 1611-1615, XP000615502 ISSN: 0027-8424 the whole document	1-64
X	WO 93 00425 A (INST MEDICAL W & E HALL) 7 January 1993 (1993-01-07) the whole document	1-64
X	SAJJADI ET AL.: "Identification of a new eph-related receptor tyrosine kinase gene from mouse and chicken that is developmentally regulated and encodes at least two forms of the receptor." NEW BIOL., vol. 3, 1991, pages 769-778, XP000920929 the whole document	3-8, 10-64
Y	LACKMANN M. ET AL: "Distinct subdomains of the EphA3 receptor mediate ligand bindin and receptor dimerization." JOURNAL OF BIOLOGICAL CHEMISTRY, (7 AUG 1998) 273/32 (20228-20237). , XP000914515 the whole document	1-64
Y	LI Y Y ET AL: "IL-1 beta alters the expression of the receptor tyrosine kinase gene r-EphA3 in neonatal rat cardiomyocytes." AMERICAN JOURNAL OF PHYSIOLOGY, (1998 JAN) 274 (1 PT 2) H331-41. , XP000913942 the whole document	1-64
P,Y	DOTTORI M. ET AL: "Cloning and characterization of EphA3 (Hek) gene promoter: DNA methylation regulates expression in hematopoietic tumor cells." BLOOD, (1 OCT 1999) 94/7 (2477-2486). , XP000907581 the whole document	1-64

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	A.H. ZISCH ET AL.: "Complex formation between EphB2 and Src requires phosphorylation of tyrosine 611 in the EphB2 juxtamembrane region." ONCOGENE, vol. 16, no. 20, 21 May 1998 (1998-05-21), pages 2657-2670, XP000913940 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/04326

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9300425 A	07-01-1993	AU 655299 B	15-12-1994
		EP 0590030 A	06-04-1994
		JP 6508747 T	06-10-1994
		NZ 243252 A	27-11-1995
		US 5674691 A	07-10-1997
		US 6020306 A	01-02-2000

09/913756

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PATENT COOPERATION TREATY

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REC'D 15 JUN 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
L0461/7057WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04326	18/02/2000	22/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant LUDWIG INSTITUTE FOR CANCER RESEARCH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/08/2000	13.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chavanne, F Telephone No. +49 89 2399 8399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/04326

I. Basis f the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-55	as originally filed	

Claims, No.:

16 (part),17-64	as originally filed	
1-15,16 (part)	with telefax of	15/02/2001

Drawings, sheets:

1/9-9/9	as originally filed

Sequence listing part of the description, pages:

1-27, filed with the letter of 15.08.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such stat ment

1. Statement

Novelty (N)	Yes:	Claims	1-64
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-4, 9, 46-49, 62-64
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	29-42

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

IV. Lack of unity of inv ntion

1. The problem underlying claims 1-61 can be regarded as the provision of EphA3 peptides which bind HLA molecules, and the nucleic acid molecule encoding said peptides, whereas the problem underlying claims 62-64 can be seen in the provision of a method for identifying genes encoding antigens presented by MHC class II molecules.
   Thus, these two problems are totally different from one another. Correspondingly, the subject-matter of claims 1-61 and 62-64 are not linked by a single inventive concept. Therefore, claims 1-61 and 62-64 lack unity a priori (Rule 13(1) PCT).

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: The journal of biological chemistry
   Vol. 267, No. 5, pp. 3262-3267, 1992

2. The closest prior art to evaluate the inventiveness of the present application is D1. D1 describes the isolation and characterisation of the EphA3 protein, a human tumour-associated protein tyrosine kinase (abstract). D1 also discloses a monoclonal antibody specific for said EphA3 protein (figures 1-3).
   The subject-mater of claims 1, 3 and 4 refers to an isolated EphA3 peptide comprising a fragment of an amino acid sequence selected from notably the EphA3 sequence, wherein said isolated peptide does not consist of any of the known full-length EphA3 proteins. Claim 1 tries to further define said peptide in that it binds an HLA class II molecule ("HLA class II-binding", "which binds an HLA class II molecule"). The same applies to claim 9 which tries to further define said EphA3 peptide in that it binds an HLA class I molecule.
   The scope of a claim referring to a peptide comprising a fragment of a protein, or to variants of a fragment of a protein, comprising e.g. additions, encompasses said protein deleted of one or a few amino acids or small fragments. The isolated

protein EphA3 is known in the art (see D1). D1 also shows that said EphA3 proteins contains antigenic determinants. Thus, the man skilled in the art by applying basic common knowledge and commonly used technics would come to the subject-matter of claims 1-4, 9 and 46-48. Thus, said subject-matter is not inventive.
The subject-matter of claim 49 further differs from D1 in that D1 does not disclose any Fab or $F(ab')_2$ fragment. However, the enzymatic digestion of a known antibody to prepare a Fab or $F(ab')_2$ fragment of said antibody is well-known in the art and commonly applied. The man skilled in the art, aware of D1, by further applying common knowledge and routinely used technics would automatically come to the subject-matter of claim 49. Thus, said subject-matter is not inventive. Therefore, claims 1-4, 9 and 46-49 does not meet the requirements of Article 33(3) PCT.

3. The subject-matter of claims 62-64 refers to a method for identifying genes encoding antigens presented by MHC class II molecules. Such methods based on the cotransfection of a cDNA library are known in the art. Thus, by applying common knowledge, the man skilled in the art would come to the subject-matter of claims 62-64. Therefore, claims 62-64 do not meet the requirements of Article 33(3) PCT.

VIII. Certain observations on the international application

1. Claims 1, 3 and 9 which refer to a peptide attempts to define the subject-matter in terms of a result to be achieved ("HLA class II-binding", "which binds an HLA class II molecule", "HLA class I-binding", "which binds an HLA class I molecule"). Such a definition is only allowable in case the invention can only be defined in such terms. However, this prerequisite is not met by the instant case since a peptide is a chemical compound which has to be characterised by structural features e.g. its amino acid sequence. Therefore, claims 1, 3 and 9 do not meet the requirements of Article 6 PCT (see also Guidelines C-III, 4.7 PCT).

2. The formulations "comprising" or "comprises" in claims 1, 3-5, 9 and 16 do not clearly define the scope of the claim. Thus, these expressions should be replaced

with "consisting of" or "consists of", respectively (Article 6 PCT).

3. Claims 1, 2, 9, 22, 23, 26, 28, 30, 34, 37, 39, 42, 44, 51, 53 and 61 lack clarity due to the formulation "a fragment of an amino acid sequence". Said formulation encompasses everything between a single amino acid and the whole sequence but one amino acid. Thus, said formulation is not adapted to clearly define the scope of these claims (Article 6 PCT). This also applies to the formulation "a fragment of a nucleotide sequence" of claims 16.

4. The term "variant" in claims 1, 2, 4, 9, 12, 23, 26, 28, 30, 34, 39, 42, 43, 51, 53, 60 and 61 is vague and not clear, it does not refer to any technical feature, and can be subject to interpretation. Moreover, the function of a peptide is not determined by its capacity of binding HLA molecules. Thus, the concept of functional variant of an EphA3 HLA class I or class II binding peptide is unclear (Article 6 PCT).

5. The scope of newly filed claims 1, 3 and 9 is limited by a disclaimer. However, it appears that the use of said disclaimer could be avoided by the clear and concise definition of the claimed peptide in positive terms (Article 6 PCT, see also Guidelines, III-4.12).

6. The term "portion" in claims 6, 14, 24, 31, 35 and 40 is vague and not clear, it does not refer to any technical feature, and can be subject to interpretation. This term does not clearly define the scope of the claims (Article 6 PCT).

7. For the assessment of the present claims 29-42 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

CLAIMS

1. An isolated EphA3 HLA class II-binding peptide comprising a fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 which binds an HLA class II molecule, or a functional variant thereof comprising one or more amino acid additions, substitutions or deletions, wherein the isolated EphA3 HLA class II-binding peptide does not consist of any of SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

2. The isolated HLA class II-binding peptide of claim 1, wherein the isolated peptide consists of a fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7, or a functional variant thereof.

3. An isolated EphA3 HLA class II-binding peptide comprising the amino acid sequence of SEQ ID NO:53, or a functional variant thereof which binds HLA class II molecules comprising one or more amino acid additions, substitutions or deletions, wherein the isolated EphA3 HLA class II-binding peptide does not consist of any of SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

4. The isolated HLA class II-binding peptide of claim 3 wherein the isolated peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:62, fragments thereof, and functional variants thereof.

5. The isolated HLA class II-binding peptide of claim 1 or claim 3, wherein the isolated peptide comprises an endosomal targeting signal.

6. The isolated HLA class II-binding peptide of claim 5, wherein the endosomal targeting signal comprises an endosomal targeting portion of a polypeptide selected from the group consisting of human invariant chain Ii and LAMP-1.

7. The isolated HLA class II-binding peptide of claim 1 or claim 3 wherein the isolated peptide is non-hydrolyzable.

8. The isolated HLA class II-binding peptide of claim 7 wherein the isolated peptide is selected from the group consisting of peptides comprising D-amino acids, peptides comprising a -psi[CH_2NH]-reduced amide peptide bond, peptides comprising a -psi[$COCH_2$]-ketomethylene peptide bond, peptides comprising a -psi[CH(CN)NH]-(cyanomethylene)amino peptide bond, peptides comprising a -psi[$CH_2CH(OH)$]-hydroxyethylene peptide bond, peptides comprising a -psi[CH_2O]-peptide bond, and peptides comprising a -psi[CH_2S]-thiomethylene peptide bond.

9. An isolated EphA3 HLA class I-binding peptide comprising a fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 which binds an HLA class I molecule, or a functional variant thereof comprising one or more amino acid additions, substitutions or deletions, wherein the isolated EphA3 HLA class I-binding peptide does not consist of any of SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

10. A composition comprising an isolated EphA3 HLA class I-binding peptide and an isolated EphA3 HLA class II-binding peptide.

11. The composition of claim 10, wherein the EphA3 HLA class I-binding peptide and the EphA3 HLA class II-binding peptide are combined as a polytope polypeptide.

12. The composition of claim 10, wherein the isolated EphA3 HLA class II-binding peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:62, fragments thereof, and functional variants thereof.

13. The composition of claim 10, wherein the isolated EphA3 HLA class II-binding peptide comprises an endosomal targeting signal.

14. The composition of claim 13, wherein the endosomal targeting signal comprises an endosomal targeting portion of a polypeptide selected from the group consisting of human invariant chain Ii and LAMP-1.

15. An isolated nucleic acid encoding a peptide selected from the group consisting of the peptide of any of claims 1-6 or 9, wherein the nucleic acid does not encode full length EphA3.

16. The isolated nucleic acid of claim 15, wherein the nucleic acid comprises a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4,